

CLAIMS

1) Recombinant baculovirus constituting an expression vector that can be used for the production of immunoglobulins in an insect cell, and characterized in that it comprises:

- an expression cassette comprising a sequence coding for at least one part of an immunoglobulin H chain, which sequence is placed under transcriptional control of a first baculovirus promoter,

- an expression cassette comprising a sequence coding for at least one part of an immunoglobulin L chain, which sequence is placed under transcriptional control of a second baculovirus promoter;

the first and the second promoters are two different promoters and located at two different loci.

2) Recombinant baculovirus in accordance with Claim 1, characterized in that one of the promoters is located at the site occupied in the wild baculovirus by the polyhedrin promoter and that the other promoter is located at the site occupied in the wild baculovirus by the p10 promoter.

3) Recombinant baculovirus in accordance with Claim 1 or 2, characterized in that the two promoters are strong promoters.

4) Recombinant baculovirus in accordance with Claim 3, characterized in that at least one of the promoters is selected from the group constituted by:

- the p10 promoter;
- the polyhedrin promoter;
- a synthetic promoter, referred to as Syn promoter and constituted by a double-strand DNA fragment the sequence of which, shown in the attached sequence listing as SEQ ID NO.: 1 and SEQ ID NO.: 2, is the following:

5- ATCAAATAAATAAGTATTTTAAAGAATTCGTACGTATTTTGTATATTAATTAATAAATACTATACTGTAAATAGATCG -3
TAGTTTATTTATTCATAAAATTTCTTAAGCATGCATAAAACATATAATTAATTTTATGATATGACATTTATCTAGCCTAG -5
3-

Sub B2
5) Recombinant baculovirus in accordance with one of Claims 1 to 4, characterized in that each expression cassette comprises: (i) a strong baculovirus promoter and, under the control of the said promoter: (ii) a sequence coding for a signal peptide; (iii) a sequence coding for a variable immunoglobulin domain; (iv) a sequence coding for a constant domain of an immunoglobulin H or L chain.

6) Recombinant baculovirus in accordance with Claim 5, characterized in that the sequence coding for a signal peptide placed under the control of the first promoter is different from the sequence coding for a signal peptide placed under the control of the second promoter.

Sub B3
7) Recombinant baculovirus in accordance with Claim 5 or 6, characterized in that at least one of the sequences coding for a signal peptide codes for a peptide that has an His-Val-Ser signal immediately upstream of the cleavage site used by the signal peptidase.

8) Recombinant baculovirus in accordance with one of Claims 5 to 7, characterized in that the sequence coding for the constant immunoglobulin domain is a sequence of human origin.

Sub B4
9) Insect cell infected by a recombinant baculovirus in accordance with one of Claims 1 to 8.

10) Procedure for the preparation of an immunoglobulin, characterized in that insect cells in accordance with Claim 9 are cultured and that the said immunoglobulin is extracted from the culture medium.

11) Immunoglobulin, characterized in that it can be obtained by the procedure in accordance with Claim 10.

~~12) Procedure for the preparation of a recombinant baculovirus in accordance with one of Claims 1 to 8, which procedure is characterized in that:~~

~~- one prepares a first transfer plasmid comprising a sequence coding for at least one part of an immunoglobulin H chain, under transcriptional control of a first strong baculovirus promoter;~~

~~- one prepares a second transfer plasmid comprising the sequence coding for at least one part of an immunoglobulin L chain, under transcriptional control of a second strong promoter of the said baculovirus;~~

~~- with the first and second promoters being two different promoters;~~

~~- one carries out the homologous recombination of the two plasmids with baculovirus DNA;~~

~~- after replication of the viral DNA in transfected cells, one proceeds to the selection of the recombinant baculoviruses that have integrated the sequence coding for at least one part of the immunoglobulin H chain and the sequence coding for at least one part of the immunoglobulin L chain.~~

~~13) Procedure in accordance with Claim 12, characterized in that each transfer plasmid used carries an insert comprising:~~

~~- an expression cassette such as defined in Claim 5 and, on both sides of this cassette, baculovirus sequences homologous with those of the regions flanking the portion of the viral genome which it is the intention to replace by insertion of the said cassette.~~

~~14) Procedure in accordance with Claim 13, characterized in that the said baculovirus sequences are homologous with those of the regions flanking the p10 gene or homologous with those of the regions flanking the polyhedrin gene.~~

~~15) Procedure in accordance with Claim 14, characterized in that the baculovirus DNA with which is effected the homologous recombination of the~~

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transfer plasmids is constituted by DNA from a baculovirus that has previously been modified by insertion of two Bsu36I on both sides of the sequence coding for the p10 protein (these two sites being the only sites for the enzyme under consideration in the genome of the said modified baculovirus) and digested by the enzyme Bsu36I.